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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>7</sup> :</b> <b>G01N 33/53, 33/566</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 00/34783</b> <b>(43) International Publication Date:</b> 15 June 2000 (15.06.00)
<b>(21) International Application Number:</b> PCT/US99/28941 <b>(22) International Filing Date:</b> 7 December 1999 (07.12.99)  <b>(30) Priority Data:</b> 09/297,493 8 December 1998 (08.12.98) US  <b>(71) Applicant:</b> SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US).  <b>(72) Inventors:</b> BERGSMA, Derk, J.; 271 Irish Road, Berwyn, PA 19312 (US). HALSEY, Wendy, S.; 435 Bayard Road, Kennett Square, PA 19348 (US). SATHE, Ganesh; 107 Hunters Run, King of Prussia, PA 19406 (US). WANG, Da-Yuan; 616 Lanmore Avenue, Wayne, PA 19087 (US). CULP, Jeffrey, S.; 482 Reginald Lane, Collegeville, PA 19426 (US).  <b>(74) Agents:</b> HECHT, Elizabeth, J. et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).		<b>(81) Designated States:</b> JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> METHODS OF SCREENING FOR AGONISTS AND ANTAGONISTS OF THE HDPXU17 RECEPTOR  <b>(57) Abstract</b>  An isolated polynucleotide encoding a human PY purinergic receptor and the protein encoded thereby are disclosed. Also disclosed is an assay for identifying agonists and antagonists of the disclosed receptor.		

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## METHODS OF SCREENING FOR AGONISTS AND ANTAGONISTS OF THE HDPXU17 RECEPTOR

### FIELD OF INVENTION

5           This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to G-protein coupled receptor family, hereinafter referred to as HDPXU17 receptor (also known as the P2Y-like receptor). The invention also relates to inhibiting or activating the action of such  
10   polynucleotides and polypeptides.

### BACKGROUND OF THE INVENTION

          It is well established that many medically significant biological processes are mediated by proteins participating in signal transduction pathways that involve G-proteins and/or second  
15   messengers, *e.g.*, cAMP (Lefkowitz, *Nature*, 1991, 351:353-354). Herein, these proteins are referred to as proteins participating in pathways with G-proteins or PPG proteins. Some examples of these proteins include the GPC receptors, such as those for adrenergic agents and dopamine (Kobilka, B.K., *et al.*, *Proc. Natl Acad. Sci., USA*, 1987, 84:46-50; Kobilka, B.K., *et al.*, *Science*, 1987, 238:650-656; Bunzow, J.R., *et al.*, *Nature*, 1988, 336:783-787), G-proteins  
20   themselves, effector proteins, *e.g.*, phospholipase C, adenylyl cyclase, and phosphodiesterase, and actuator proteins, *e.g.*, protein kinase A and protein kinase C (Simon, M.I., *et al.*, *Science*, 1991, 252:802-8).

          For example, in one form of signal transduction, the effect of hormone binding is activation of the enzyme, adenylyl cyclase, inside the cell. Enzyme activation by hormones is  
25   dependent on the presence of the nucleotide, GTP. GTP also influences hormone binding. A G-protein connects the hormone receptor to adenylyl cyclase. G-protein was shown to exchange GTP for bound GDP when activated by a hormone receptor. The GTP-carrying form then binds to activated adenylyl cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a dual role, as an  
30   intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

The membrane protein gene superfamily of G-protein coupled receptors has been characterized as having seven putative transmembrane domains. The domains are believed to represent transmembrane  $\alpha$ -helices connected by extracellular or cytoplasmic loops. G-protein coupled receptors include a wide range of biologically active receptors, such as hormone, viral, growth factor and neuroreceptors.

G-protein coupled receptors (otherwise known as 7TM receptors) have been characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. The G-protein family of coupled receptors includes dopamine receptors which bind to neuroleptic drugs used for treating psychotic and neurological disorders. Other examples of members of this family include, but are not limited to, calcitonin, adrenergic, endothelin, cAMP, adenosine, muscarinic, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsins, endothelial differentiation gene-1, rhodopsins, odorant, and cytomegalovirus receptors.

Most G-protein coupled receptors have single conserved cysteine residues in each of the first two extracellular loops which form disulfide bonds that are believed to stabilize functional protein structure. The 7 transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6, and TM7. TM3 has been implicated in signal transduction.

Phosphorylation and lipidation (palmitoylation or farnesylation) of cysteine residues can influence signal transduction of some G-protein coupled receptors. Most G-protein coupled receptors contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several G-protein coupled receptors, such as the  $\beta$ -adrenoreceptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

For some receptors, the ligand binding sites of G-protein coupled receptors are believed to comprise hydrophilic sockets formed by several G-protein coupled receptor transmembrane domains, said sockets being surrounded by hydrophobic residues of the G-protein coupled receptors. The hydrophilic side of each G-protein coupled receptor transmembrane helix is postulated to face inward and form a polar ligand binding site. TM3 has been implicated in several G-protein coupled receptors as having a ligand binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine and TM6 or TM7 phenylalanines or tyrosines are also implicated in ligand binding.

G-protein coupled receptors can be intracellularly coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels and transporters (*see, Johnson, et al., Endoc. Rev.,*

1989, 10:317-331). Different G-protein  $\alpha$ -subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of G-protein coupled receptors has been identified as an important mechanism for the regulation of G-protein coupling of some G-protein coupled receptors. G-protein coupled receptors are found in numerous sites within a mammalian host. Over the past 15 years, nearly 350 therapeutic agents targeting 7 transmembrane (7 TM) receptors have been successfully introduced onto the market.

This indicates that these receptors have an established, proven history as therapeutic targets. Clearly, there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to: infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; benign prostatic hypertrophy; leukopenia; malignant lymphomas; Hodgkin's disease; leukemias; leukocytosis; lymphadenitis; myeloproliferative disorders; myelodysplastic syndromes; myelomas; macroglobulinemia; heavy-chain disease; Langerhan's cell histiocytosis; splenomegaly; anemias; bleeding disorders; chronic obstructive pulmonary disease (COPD)-emphysema; allergies; bronchitis; asthma; bronchiectasis; adult respiratory distress syndrome; pleural effusions; pneumoconioses; sarcoidosis; systemic lupus erythematosus; Sjögren's syndrome; scleroderma; inflammatory myopathies; mixed connective tissue disease; amyloidosis; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome.

## **25 SUMMARY OF THE INVENTION**

In one aspect, the invention relates to HDPXU17 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such HDPXU17 polypeptides and polynucleotides. Such uses include the treatment of: infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; benign prostatic hypertrophy; leukopenia; malignant lymphomas; Hodgkin's disease; leukemias; leukocytosis; lymphadenitis; myeloproliferative disorders;

myelodysplastic syndromes; myelomas; macroglobulinemia; heavy-chain disease; Langerhan's cell histiocytosis; splenomegaly; anemias; bleeding disorders; chronic obstructive pulmonary disease (COPD)-emphysema; allergies; bronchitis; asthma; bronchiectasis; adult respiratory distress syndrome; pleural effusions; pneumoconioses; sarcoidosis; systemic lupus  
5 erythematosus; Sjögren's syndrome; scleroderma; inflammatory myopathies; mixed connective tissue disease; amyloidosis; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome., among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials  
10 provided by the invention, and treating conditions associated with HDPXU17 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate HDPXU17 activity or levels.

In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with  
15 HDPXU17 imbalance with the identified compounds. In particular, the preferred method for identifying agonist or antagonist of HDPXU17 receptor of the present invention comprises:

contacting a cell expressing on the surface thereof the receptor, said receptor being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said receptor, with a compound to be screened under conditions to  
20 permit binding to the receptor; and

determining whether the compound binds to and activates or inhibits the receptor by measuring the level of a signal generated from the interaction of the compound with the receptor.

In a further preferred embodiment, the method further comprises conducting the identification of agonist or antagonist in the presence of labeled or unlabeled deoxyadenosine  
25 triphosphate (hereinafter referred to as "dATP"), deoxyadenosine monophosphate (hereinafter referred to as "dAMP"), or uridine triphosphate (hereinafter referred to as "UTP").

In another embodiment of the method for identifying agonist or antagonist of a HDPXU17 receptor of the present invention comprises:

determining the inhibition of binding of a ligand to cells which have the receptor on the  
30 surface thereof, or to cell membranes containing the receptor, in the presence of a candidate compound under conditions to permit binding to the receptor, and determining the amount of ligand bound to the receptor, such that a compound capable of causing reduction of binding of a

ligand is an agonist or antagonist. Preferably, the ligand is dATP, dAMP, or UTP. Yet more preferably, dATP, dAMP, or UTP is labeled.

Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate HDPXU17 activity or levels.

## 5 DESCRIPTION OF THE INVENTION

### Definitions

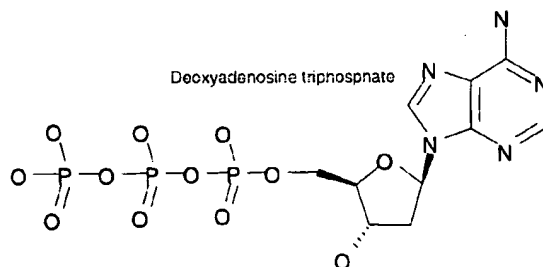
The following definitions are provided to facilitate understanding of certain terms used frequently herein.

10 "HDPXU17" refers, among others, to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, or an allelic variant thereof.

"Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said HDPXU17 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said HDPXU17.

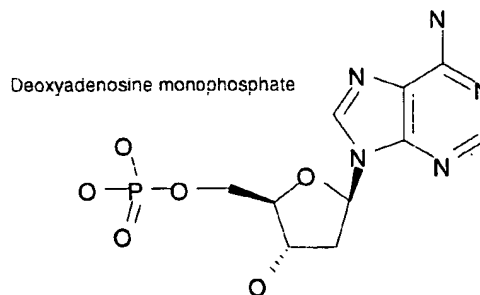
15 "HDPXU17 gene" refers to a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 or allelic variants thereof and/or their complements.

"dATP" refers to deoxyadenosine triphosphate, which has the following structure:

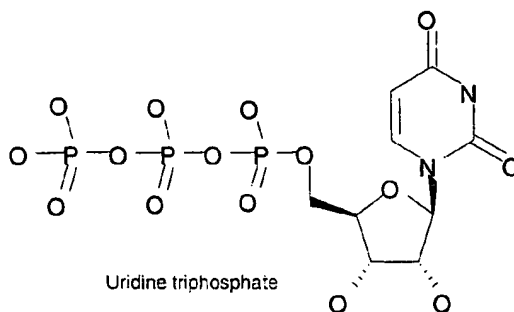


"dAMP" refers to deoxyadenosine monophosphate, which has the following structure:

20



"UTP" refers to uridine triphosphate, which has the following structure:



5            "Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

          "Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original  
10        environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

          "Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides"  
15        include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or  
20        DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of  
25        polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.



"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.*, peptide isosteres.

"Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter, *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth. Enzymol.* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A

typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., *et al.*, *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. *et al.*, *J. Molec. Biol.* 215: 403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., *et al.*, NCBI NLM NIH Bethesda, MD

20894; Altschul, S., *et al.*, *J. Mol. Biol.* 215: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

Preferred parameters for polypeptide sequence comparison include the following:

1) Algorithm: Needleman and Wunsch, *J. Mol. Biol.* 48: 443-453 (1970)

5 Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA.* 89:10915-10919 (1992)

Gap Penalty: 12

Gap Length Penalty: 4

10 A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps).

Preferred parameters for polynucleotide comparison include the following:

1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

15 Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

20 A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polynucleotide comparisons.

Preferred polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide having at least a 50,60, 70, 80, 85, 90, 95, 97 or 100% identity to a polynucleotide reference sequence of SEQ ID NO:1, wherein said reference sequence may be  
25 identical to the sequence of SEQ ID NO: 1 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal  
30 positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the numerical percent of the respective percent identity and subtracting that product

from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \bullet y),$$

- 5 wherein  $n_n$  is the number of nucleotide alterations,  $x_n$  is the total number of nucleotides in SEQ ID NO:1, and  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and wherein any non-integer product of  $x_n$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_n$ . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create  
 10 nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

- Preferred polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50,60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide  
 15 reference sequence of SEQ ID NO:2, wherein said reference sequence may be identical to the sequence of SEQ ID NO: 2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the  
 20 amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the numerical percent of the respective percent identity and  
 25 subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \bullet y),$$

- wherein  $n_a$  is the number of amino acid alterations,  $x_a$  is the total number of amino acids in  
 30 SEQ ID NO:2, and  $y$  is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and wherein any non-integer product of  $x_a$  and  $y$  is rounded down to the nearest integer prior to subtracting it from  $x_a$ .

### Polypeptides of the Invention

In one aspect, the present invention relates to HDPXU17 polypeptides (or HDPXU17 proteins). The HDPXU17 polypeptides include the polypeptide of SEQ ID NO:2; as well as polypeptides comprising the amino acid sequence of SEQ ID NO:2; and polypeptides  
5 comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within HDPXU17 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid  
10 sequence of SEQ ID NO: 2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Preferably HDPXU17 polypeptides exhibit at least one biological activity of the receptor.

The HDPXU17 polypeptides may be in the form of the "mature" protein or may be a  
15 part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the HDPXU17 polypeptides are also included in the invention. A  
20 fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned HDPXU17 polypeptides. As with HDPXU17 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example,  
25 fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of HDPXU17 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of HDPXU17 polypeptides, except for deletion of a continuous series of residues that  
30 includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions,

beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the receptor, including antigenic activity. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- *i.e.*, those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The HDPXU17 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

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#### **Polynucleotides of the Invention**

Another aspect of the invention relates to HDPXU17 polynucleotides. HDPXU17 polynucleotides include isolated polynucleotides which encode the HDPXU17 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, HDPXU17 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding a HDPXU17 polypeptide of SEQ ID NO: 2, and polynucleotide having the particular sequence of SEQ ID NO:1. HDPXU17 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the HDPXU17 polypeptide of SEQ ID NO:2, and a polynucleotide comprising a nucleotide sequence that is at least 80% identical to that of SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most

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highly preferred, with at least 99% being the most preferred. Also included under HDPXU17 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are

5 complementary to such HDPXU17 polynucleotides.

HDPXU17 of the invention is structurally related to other proteins of the G-protein coupled receptor family, as shown by the results of sequencing the cDNA of Table 1 (SEQ ID NO:1) encoding human HDPXU17. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 89 to 1210) encoding a polypeptide of 374 amino acids (SEQ

10 ID NO:2). The amino acid sequence of Table 2 (SEQ ID NO:2) has about 98 % identity (using FASTA) in 374 amino acid residues with human P2Y11 receptor (Accession # AF030335, Communi, D., *et al.*, *J. Biol. Chem.* 1997, in press). Furthermore, HDPXU17 (SEQ ID No. 2) is 31.9 % identical to human P2Y1 purinergic receptor over 254 amino acid residues (Accession # P47900, Ayyanathan, K., *et al.*, *Biochem. Biophys. Res. Commun.* 218, 3, 783-788, 1996). The

15 nucleotide sequence of Table 1 (SEQ ID NO:1) has about 98% identity (using BLAST) in 110 nucleotide residues with Homo sapiens cDNA clone hbc240 (Accession # T10449, Takeda, J., *et al.*, *Hum. Mol. Genet.* 2, 1793-1798, 1993). Furthermore, HDPXU17 (SEQ ID NO:1) is 99.14 % identical to Soares Ovary tumor NbHOT *homo sapiens* cDNA over 116 nucleotide residues (Accession # AA405284, Hillier, L., *et al.*, WashU-Merck EST project, 1997, Unpublished).

20 Thus, HDPXU17 polypeptides and polynucleotides of the present invention are expected to have, *inter alia*, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

TABLE 1

25	1	CTGGGGCAGG	CCCCACTAAG	CCACTGGTGA	CTGGGGGAGG	GGCTGGGGAA
	51	CTGGGTAGCA	GACACAGGCT	GAGGATCGGC	ACGGGAGCAT	GGCAGCCAAAC
	101	GTCTCGGGTG	CCAAGTCCTG	CCCTGCCAAC	TTCTTGGCAG	CTGCCGACGA
30	151	CAAACTCAGT	GGGTTCCAGG	GGGACTTCCT	GTGGCCCATA	CTGGTGGTTG
	201	AGTTCCTGGT	GGCCGTGGCC	AGCAATGGCC	TGGCCCTGTA	CCGCTTCAGC
	251	ATCCGGAAGC	AGCGCCCATG	GCACCCCGCC	GTGGTCTTCT	CTGTCCAGCT
35	301	GGCAGTCAGC	GACCTGCTCT	GCGCCCTGAC	GCTGCCCCCG	CTGGCCGCCT
	351	ACCTCTATCC	CCCCAAGCAC	TGGCGCTATG	GGGAGGCCGC	GTGCCGCCTG
40	401	GAGCGCTTCC	TCTTCACCTG	CAACCTGCTG	GGCAGCGTCA	TCTTCATCAC
	451	CTGCATCAGC	CTCAACCGCT	ACCTGGGCAT	CGTGCACCCC	TTCTTCGCCC

501 GAAGCCACCT GCGACCCAAG CACGCCTGGG CCGTGAGCGC TGCCGGCTGG  
 551 GTCCTGGCCG CCCTGCTGGC CATGCCCACA CTCAGCTTCT CCCACCTGAA  
 5 601 GAGGCCGAG CAGGGGGCGG GCAACTGCAG CGTGGCCAGG CCCGAGGCCT  
 651 GCATCAAGTG TCTGGGGACA GCAGACCACG GGCTGGCGGC CTACAGAGCG  
 10 701 TATAGCCTGG TGCTGGCGGG GTTGGGCTGC GGCCTGCCGC TGCTGCTCAC  
 751 GCTGGCAGCC TACGGCGCCC TCGGGCGGGC CGTGCTACGC AGCCCAGGCA  
 801 TGACTGTGGC CGAGAAGCTG CGTGTGGCAG CGTTGGTGGC CAGTGGTGTG  
 15 851 GCCCTCTACG CCAGCTCCTA TGTGCCCTAC CACATCATGC GGGTGCTCAA  
 901 CGTGGATGCT CGGCGGCGCT GGAGCACCCG CTGCCCCAGC TTTGCAGACA  
 951 TAGCCCAGGC CACAGCAGCC CTGGAGCTGG GGCCCTACGT GGGCTACCAG  
 20 1001 GTGATGCGGG GCCTCATGCC CCTGGCCTTC TGTGTCCACC CTCTACTCTA  
 1051 CATGGCCGCA GTGCCCAGCC TGGGCTGCTG CTGCCGACAC TGCCCCGGCT  
 1101 ACAGGGACAG CTGGAACCCA GAGGACGCCA AGAGCACTGG CCAAGCCCTG  
 25 1151 CCCCTCAATG CCACAGCCGC CCCTAAACCG TCAGAGCCCC AGTCCCGTGA  
 1201 GCTGAGCCAA TGATGTGGCC TAGCGGAAGC TGCCTCCTCA CCCTAGGTGT  
 30 1251 TGCTGGAGAA CCCTGAGGGC AGGGCCCGAG CCCCAGACACA TCCCTTCCCC  
 1301 CAAAAAGCAA CACCTGTGCT TGCAGCCAGG TCAGGCCAG C-TGCAGCCCA  
 35 1351 GGCAGGAGCA GTCGCCTTTC CCACCCACAG CGCTGGCCAC AGGGCTCCCT  
 1401 GCAGGGTCAG GGACCAGACC ACGCCAGAG GAGGGGAGGC ACTGGCCCCC  
 1451 GCCACAGGAC TGGAGACGCA AGAACAAAA GAACCAAGTA GAGAGAGTGG  
 40 1501 AGCTGCTTTA TTGCCCTTGG AGCCCGCGCT CTCGGAGGCT GTCTTCTGTC  
 1551 GCCAAGGGTC CCGGACCGAG TACACAGTGG CAGCTGGCTT AGTTGGTGGA  
 45 1601 CGGCCTGGGG TAGGGGAGGG TGGCAGGTAT AAGACTTCTG GGGGCACCCC  
 1651 AAGACCCAG ACACCCAAGT GGCATCTTGG GGGTGGGTGG GCAGAGGACG  
 1701 GGGTAATGTG AGGACGAAGC GGGCACGGAG CCAGATGGCC AGTCTCCAGG  
 50 1751 CCTGGTCCAC GGA CTGGCAG GGACCCAGG CACAAGAGCT GCCACCCCTC  
 1801 TGCCCGGTTT TGGAAAAAAA CAATAAAGGA CTGTCCCCTC AAAACCAGCC  
 55 1851 GGGGGACTGT TTAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA  
 1901 AAAAAAAAAA



**Table 2<sup>b</sup>**

1	MAANVSGAKS	CPANFLAAAD	DKLSGFQGDF	LWPILVVEFL	VAVASNGLAL
51	YRFSIRKQRP	WHPAVVFSVQ	LAVSDLLCAL	TLPLLAAYLY	PPKHWRGGEA
101	ACRLERFLFT	CNLLGSVIFI	TCISLNRYLG	IVHPFFARSH	LRPKHAWAVS
151	AAGWVLAALL	AMPTLSFSLH	KRPQQGAGNC	SVARPEACIK	CLGTADHGLA
201	AYRAYSLVLA	GLGCGLPLLL	TLAAYGALGR	AVLRSPGMTV	AEKLRVAALV
251	ASGVALYASS	YVPYHIMRVL	NVDARRRWST	RCPSFADIAQ	ATAALELGPY
301	VGYQVMRGLM	PLAFCVHPLL	YMAAVPSLGC	CCRHCPCGYRD	SWNPEDAKST
351	GQALPLNATA	APKPSEPQSR	ELSG		

<sup>b</sup> An amino acid sequence of a human HDPXU17 (SEQ ID NO: 2).

5 One polynucleotide of the present invention encoding HDPXU17 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human dendriatic cells using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature*, (1992) 355:632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from  
10 natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding HDPXU17 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 89 to 1210 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy  
15 (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of HDPXU17 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or  
20 secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be

encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding HDPXU17 variants comprising the amino acid sequence of HDPXU17 polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding HDPXU17 and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to the HDPXU17 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding HDPXU17 polypeptide, including homologs and orthologs from species other than human, comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Thus in another aspect, HDPXU17 polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof. Also included with HDPXU17 polypeptides are polypeptide

comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or, alternatively, conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 5 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human 10 disease.

### Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with 15 vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction 20 of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid- 25 mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells 30 such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can *e.g.*, be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, vectors derived from bacterial

plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and  
5 bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set  
10 forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL (supra)*.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

15 If the HDPXU17 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If HDPXU17 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

20 HDPXU17 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for  
25 purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

#### Diagnostic Assays

This invention also relates to the use of HDPXU17 polynucleotides for use as diagnostic  
30 reagents. Detection of a mutated form of HDPXU17 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of HDPXU17.

Individuals carrying mutations in the HDPXU17 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for  
 5 detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled HDPXU17 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched  
 10 duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. *See, e.g., Myers et al., Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method.  
 15 *See Cotton, et al., Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising HDPXU17 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of, *e.g.*, genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and  
 20 genetic variability. (See for example: M.Chee, *et al., Science*, 274:610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to: infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris;  
 25 myocardial infarction; ulcers; benign prostatic hypertrophy; leukopenia; malignant lymphomas; Hodgkin's disease; leukemias; leukocytosis; lymphadenitis; myeloproliferative disorders; myelodysplastic syndromes; myelomas; macroglobulinemia; heavy-chain disease; Langerhan's cell histiocytosis; splenomegaly; anemias; bleeding disorders; chronic obstructive pulmonary disease (COPD)-emphysema; allergies; bronchitis; asthma; bronchiectasis; adult respiratory  
 30 distress syndrome; pleural effusions; pneumoconioses; sarcoidosis; systemic lupus erythematosus; Sjögren's syndrome; scleroderma; inflammatory myopathies; mixed connective tissue disease; amyloidosis; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias,

such as Huntington's disease or Gilles de la Tourette's syndrome through detection of mutation in the HDPXU17 gene by the methods described.

In addition, infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; benign prostatic hypertrophy; leukopenia; malignant lymphomas; Hodgkin's disease; leukemias; leukocytosis; lymphadenitis; myeloproliferative disorders; myelodysplastic syndromes; myelomas; macroglobulinemia; heavy-chain disease; Langerhan's cell histiocytosis; splenomegaly; anemias; bleeding disorders; chronic obstructive pulmonary disease (COPD)-emphysema; allergies; bronchitis; asthma; bronchiectasis; adult respiratory distress syndrome; pleural effusions; pneumoconioses; sarcoidosis; systemic lupus erythematosus; Sjögren's syndrome; scleroderma; inflammatory myopathies; mixed connective tissue disease; amyloidosis; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome. can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of HDPXU17 polypeptide or HDPXU17 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an HDPXU17 polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagnostic kit for a disease or susceptibility to a disease, particularly: infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; benign prostatic hypertrophy; leukopenia; malignant lymphomas; Hodgkin's disease; leukemias; leukocytosis; lymphadenitis; myeloproliferative disorders; myelodysplastic syndromes; myelomas; macroglobulinemia; heavy-chain disease; Langerhan's cell histiocytosis; splenomegaly; anemias; bleeding disorders; chronic obstructive pulmonary disease (COPD)-emphysema; allergies; bronchitis; asthma; bronchiectasis; adult respiratory distress syndrome; pleural

effusions; pneumoconioses; sarcoidosis; systemic lupus erythematosus; Sjögren's syndrome; scleroderma; inflammatory myopathies; mixed connective tissue disease; amyloidosis; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or

5 Gilles de la Tourette's syndrome., which comprises:

(a) a HDPXU17 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof;

(b) a nucleotide sequence complementary to that of (a);

(c) a HDPXU17 polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or

(d) an antibody to a HDPXU17 polypeptide, preferably to the polypeptide of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

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#### **Chromosome Assays**

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to

20 chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library).

25 The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the

30 disease.

**Antibodies**

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the HDPXU17 polypeptides. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the HDPXU17 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, *et al.*, *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor, *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole, *et al.*, *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against HDPXU17 polypeptides may also be employed to treat: infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; benign prostatic hypertrophy; leukopenia; malignant lymphomas; Hodgkin's disease; leukemias; leukocytosis; lymphadenitis; myeloproliferative disorders; myelodysplastic syndromes; myelomas; macroglobulinemia; heavy-chain disease; Langerhan's cell histiocytosis; splenomegaly; anemias; bleeding disorders; chronic obstructive pulmonary disease (COPD)-emphysema; allergies; bronchitis; asthma; bronchiectasis; adult respiratory distress syndrome; pleural effusions; pneumoconioses; sarcoidosis; systemic lupus erythematosus; Sjögren's syndrome; scleroderma; inflammatory myopathies; mixed connective tissue disease; amyloidosis; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression,



delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome., among others.

## 5 Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with HDPXU17 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from: infections such as bacterial, fungal, protozoan and viral infections, particularly  
 10 infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; benign prostatic hypertrophy; leukopenia; malignant lymphomas; Hodgkin's disease; leukemias; leukocytosis; lymphadenitis; myeloproliferative disorders; myelodysplastic syndromes; myelomas; macroglobulinemia;  
 15 heavy-chain disease; Langerhan's cell histiocytosis; splenomegaly; anemias; bleeding disorders; chronic obstructive pulmonary disease (COPD)-emphysema; allergies; bronchitis; asthma; bronchiectasis; adult respiratory distress syndrome; pleural effusions; pneumoconioses; sarcoidosis; systemic lupus erythematosus; Sjögren's syndrome; scleroderma; inflammatory myopathies; mixed connective tissue disease; amyloidosis; and psychotic and  
 20 neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome., among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering HDPXU17 polypeptide via a vector directing expression of HDPXU17 polynucleotide *in vivo* in order to induce such an  
 25 immunological response to produce antibody to protect said animal from the aforementioned diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a HDPXU17 polypeptide wherein the composition comprises a  
 30 HDPXU17 polypeptide or HDPXU17 gene. The vaccine formulation may further comprise a suitable carrier. Since HDPXU17 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal, *etc.*, injection). Formulations suitable for parenteral administration include

aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

#### 10 Screening Assays

The HDPXU17 polypeptide of the present invention may be employed in a process for screening for compounds which bind to and activate the HDPXU17 polypeptides of the present invention (called agonists), or inhibit the interaction of the HDPXU17 polypeptides with receptor ligands (called antagonists).

15 Thus, polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics. See Coligan, *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

20 HDPXU17 proteins are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate HDPXU17 on the one hand and which can inhibit the function of HDPXU17 on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as: infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; benign prostatic hypertrophy; leukopenia; malignant lymphomas; Hodgkin's disease; leukemias; leukocytosis; lymphadenitis; myeloproliferative disorders; myelodysplastic syndromes; myelomas; macroglobulinemia; heavy-chain disease; Langerhan's cell histiocytosis; splenomegaly; anemias; bleeding disorders; chronic obstructive pulmonary disease (COPD)-emphysema; allergies; bronchitis; asthma; bronchiectasis; adult respiratory distress syndrome; pleural effusions; pneumoconioses; sarcoidosis; systemic lupus erythematosus; Sjögren's syndrome; scleroderma; inflammatory myopathies; mixed connective

tissue disease; amyloidosis; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome.

5 In general, such screening procedures involve providing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. In particular, a polynucleotide encoding the receptor of the present invention is employed to transfect cells to thereby express the HDPXU17 polypeptide. The expressed receptor is then contacted with a test compound to observe binding, stimulation or inhibition of a functional response.

10 One such screening procedure involves the use of melanophores which are transfected to express the HDPXU17 polypeptide of the present invention. Such a screening technique is described in PCT WO 92/01810, published February 6, 1992. Such an assay may be employed to screen for a compound which inhibits activation of the receptor polypeptide of the present invention by contacting the melanophore cells which encode the receptor with both the  
15 receptor ligand, such as dATP, dAMP, or UTP, and a compound to be screened. Inhibition of the signal generated by the ligand indicates that a compound is a potential antagonist for the receptor, *i.e.*, inhibits activation of the receptor.

The technique may also be employed for screening of compounds which activate the receptor by contacting such cells with compounds to be screened and determining whether such  
20 compound generates a signal, *i.e.*, activates the receptor.

Other screening techniques include the use of cells which express the HDPXU17 polypeptide (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation. In this technique, compounds may be contacted with cells expressing the receptor polypeptide of the present invention. A second messenger  
25 response, *e.g.*, signal transduction or pH changes, is then measured to determine whether the potential compound activates or inhibits the receptor.

Another screening technique involves expressing the HDPXU17 polypeptide in which the receptor is linked to phospholipase C or D. Representative examples of such cells include, but are not limited to, endothelial cells, smooth muscle cells, and embryonic kidney cells. The  
30 screening may be accomplished as hereinabove described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase second signal.

Another method involves screening for compounds which are antagonists, and thus inhibit activation of the receptor polypeptide of the present invention by determining inhibition

of binding of labeled ligand, such as dATP, dAMP, or UTP, to cells which have the receptor on the surface thereof, or cell membranes containing the receptor. Such a method involves transfecting a eukaryotic cell with DNA encoding the HDPXU17 polypeptide such that the cell expresses the receptor on its surface. The cell is then contacted with a potential antagonist in the presence of a labeled form of a ligand, such as dATP, dAMP, or UTP. The ligand can be labeled, *e.g.*, by radioactivity. The amount of labeled ligand bound to the receptors is measured, *e.g.*, by measuring radioactivity associated with transfected cells or membrane from these cells. If the compound binds to the receptor, the binding of labeled ligand to the receptor is inhibited as determined by a reduction of labeled ligand which binds to the receptors. This method is called binding assay. Naturally, this same technique can be used to look for an agonist.

Another screening procedure involves the use of mammalian cells (CHO, HEK 293, *Xenopus* Oocytes, RBL-2H3, *etc.*) which are transfected to express the receptor of interest. The cells are loaded with an indicator dye that produces a fluorescent signal when bound to calcium, and the cells are contacted with a test substance and a receptor agonist, such as dATP, dAMP, or UTP. Any change in fluorescent signal is measured over a defined period of time using, for example, a fluorescence spectrophotometer or a fluorescence imaging plate reader. A change in the fluorescence signal pattern generated by the ligand indicates that a compound is a potential antagonist or agonist for the receptor.

Another screening procedure involves use of mammalian cells (CHO, HEK293, *Xenopus* Oocytes, RBL-2H3, *etc.*) which are transfected to express the receptor of interest, and which are also transfected with a reporter gene construct that is coupled to activation of the receptor (for example, luciferase or beta-galactosidase behind an appropriate promoter). The cells are contacted with a test substance and the receptor agonist (ligand), such as dATP, dAMP, or UTP, and the signal produced by the reporter gene is measured after a defined period of time. The signal can be measured using a luminometer, spectrophotometer, fluorimeter, or other such instrument appropriate for the specific reporter construct used. Inhibition of the signal generated by the ligand indicates that a compound is a potential antagonist for the receptor.

Another screening technique for antagonists or agonists involves introducing RNA encoding the HDPXU17 polypeptide into *Xenopus* oocytes (or CHO, HEK 293, RBL-2H3, *etc.*) to transiently or stably express the receptor. The receptor oocytes are then contacted with the receptor ligand, such as dATP, dAMP, or UTP, and a compound to be screened. Inhibition or

activation of the receptor is then determined by detection of a signal, such as, cAMP, calcium, proton, or other ions.

Another method involves screening for HDPXU17 polypeptide inhibitors by determining inhibition or stimulation of HDPXU17 polypeptide-mediated cAMP and/or  
5 adenylate cyclase accumulation or diminution. Such a method involves transiently or stably transfecting a eukaryotic cell with HDPXU17 polypeptide receptor to express the receptor on the cell surface. The cell is then exposed to potential antagonists in the presence of HDPXU17 polypeptide ligand, such as dATP, dAMP, or UTP. The changes in levels of cAMP is then measured over a defined period of time, for example, by radio-immuno or protein binding  
10 assays (for example using Flashplates or a scintillation proximity assay). Changes in cAMP levels can also be determined by directly measuring the activity of the enzyme, adenyl cyclase, in broken cell preparations. If the potential antagonist binds the receptor, and thus inhibits HDPXU17 polypeptide-ligand binding, the levels of HDPXU17 polypeptide-mediated cAMP, or adenylate cyclase activity, will be reduced or increased.

15 Another screening method for agonists and antagonists relies on the endogenous pheromone response pathway in the yeast, *Saccharomyces cerevisiae*. Heterothallic strains of yeast can exist in two mitotically stable haploid mating types, MATa and MATa. Each cell type secretes a small peptide hormone that binds to a G-protein coupled receptor on opposite mating-type cells which triggers a MAP kinase cascade leading to G1 arrest as a prelude to cell  
20 fusion. Genetic alteration of certain genes in the pheromone response pathway can alter the normal response to pheromone, and heterologous expression and coupling of human G-protein coupled receptors and humanized G-protein subunits in yeast cells devoid of endogenous pheromone receptors can be linked to downstream signaling pathways and reporter genes (*e.g.*, U.S. Patents 5,063,154; 5,482,835; 5,691,188). Such genetic alterations include, but are not  
25 limited to, (i) deletion of the STE2 or STE3 gene encoding the endogenous G-protein coupled pheromone receptors; (ii) deletion of the FAR1 gene encoding a protein that normally associates with cyclin-dependent kinases leading to cell cycle arrest; and (iii) construction of reporter genes fused to the FUS1 gene promoter (where FUS1 encodes a membrane-anchored glycoprotein required for cell fusion). Downstream reporter genes can permit either a positive  
30 growth selection (*e.g.*, histidine prototrophy using the FUS1-HIS3 reporter), or a colorimetric, fluorimetric or spectrophotometric readout, depending on the specific reporter construct used (*e.g.*, b-galactosidase induction using a FUS1-LacZ reporter).

The yeast cells can be further engineered to express and secrete small peptides from random peptide libraries, some of which can permit autocrine activation of heterologously expressed human (or mammalian) G-protein coupled receptors (Broach, *et al.*, *Nature* 384: 14-16, 1996; Manfredi, *et al.*, *Mol. Cell. Biol.* 16: 4700-4709, 1996). This provides a rapid direct growth selection (*e.g.*, using the FUS1-HIS3 reporter) for surrogate peptide agonists that activate characterized or orphan receptors. Alternatively, yeast cells that functionally express human (or mammalian) G-protein coupled receptors linked to a reporter gene readout (*e.g.*, FUS1-LacZ) can be used as a platform for high-throughput screening of known ligands, fractions of biological extracts and libraries of chemical compounds for either natural or surrogate ligands. Functional agonists of sufficient potency (whether natural or surrogate) can be used as screening tools in yeast cell-based assays for identifying G-protein coupled receptor antagonists. For example, agonists will promote growth of a cell with *FUS-HIS3* reporter or give positive readout for a cell with *FUS1-LacZ*. However, a candidate compound which inhibits growth or negates the positive readout induced by an agonist is an antagonist. For this purpose, the yeast system offers advantages over mammalian expression systems due to its ease of utility and null receptor background (lack of endogenous G-protein coupled receptors) which often interferes with the ability to identify agonists or antagonists.

The present invention also provides a method for identifying new ligands not known to be capable of binding to an HDPXU17 polypeptides. The screening assays described above for identifying agonists may be used to identify new ligands.

The present invention also contemplates agonists and antagonists obtainable from the above described screening methods.

Examples of potential HDPXU17 polypeptide receptor antagonists include peptidomimetics, synthetic organic molecules, natural products, antibodies, *etc.*, which bind to the receptor, but do not elicit a second messenger response, such that the activity of the receptor is prevented.

Potential antagonists also include proteins which are closely related to the ligand of the HDPXU17 polypeptide receptor, *i.e.*, a fragment of the ligand, which have lost biological function, and when they bind to the HDPXU17 polypeptide receptor, elicit no response.

Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, and ligands for HDPXU17 polypeptides, which comprises:

(a) a HDPXU17 polypeptide, preferably that of SEQ ID NO:2; and further preferably comprises labeled or unlabeled dATP, dAMP, or UTP;

(b) a recombinant cell expressing a HDPXU17 polypeptide, preferably that of SEQ ID NO:2; and further preferably comprises labeled or unlabeled dATP, dAMP, or UTP; or

(c) a cell membrane expressing HDPXU17 polypeptide; preferably that of SEQ ID NO: 2; and further preferably comprises labeled or unlabeled dATP, dAMP, or UTP.

5 It will be appreciated that in any such kit, (a), (b), or (c) may comprise a substantial component.

As noted above, a potential antagonist is a small molecule which binds to the HDPXU17 polypeptide receptor, making it inaccessible to ligands such that normal biological activity is prevented. Examples of small molecules include, but are not limited to, small  
10 peptides or peptide-like molecules.

Potential antagonists also include soluble forms of HDPXU17 polypeptide receptor, *e.g.*, fragments of the receptor, which bind to the ligand and prevent the ligand from interacting with membrane bound HDPXU17 polypeptide receptors.

The HDPXU17 polypeptide of the present invention may be employed in a  
15 screening process for compounds which bind the receptor and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural  
20 substrates and ligands or may be structural or functional mimetics. See Coligan, *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

HDPXU17 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate HDPXU17 on the one hand and which can inhibit the function of HDPXU17 on the other hand. In general,  
25 agonists are employed for therapeutic and prophylactic purposes for such conditions as : infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; benign prostatic hypertrophy; leukopenia; malignant lymphomas;  
30 Hodgkin's disease; leukemias; leukocytosis; lymphadenitis; myeloproliferative disorders; myelodysplastic syndromes; myelomas; macroglobulinemia; heavy-chain disease; Langerhan's cell histiocytosis; splenomegaly; anemias; bleeding disorders; chronic obstructive pulmonary

disease (COPD)-emphysema; allergies; bronchitis; asthma; bronchiectasis; adult respiratory distress syndrome; pleural effusions; pneumoconioses; sarcoidosis; systemic lupus erythematosus; Sjögren's syndrome; scleroderma; inflammatory myopathies; mixed connective tissue disease; amyloidosis; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome.

Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as: infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; benign prostatic hypertrophy; leukopenia; malignant lymphomas; Hodgkin's disease; leukemias; leukocytosis; lymphadenitis; myeloproliferative disorders; myelodysplastic syndromes; myelomas; macroglobulinemia; heavy-chain disease; Langerhan's cell histiocytosis; splenomegaly; anemias; bleeding disorders; chronic obstructive pulmonary disease (COPD)-emphysema; allergies; bronchitis; asthma; bronchiectasis; adult respiratory distress syndrome; pleural effusions; pneumoconioses; sarcoidosis; systemic lupus erythematosus; Sjögren's syndrome; scleroderma; inflammatory myopathies; mixed connective tissue disease; amyloidosis; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome.

In general, such screening procedures involve producing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response.

One screening technique includes the use of cells which express receptor of this invention (for example, transfected CHO cells) in a system which measures extracellular pH or intracellular calcium changes caused by receptor activation. In this technique, compounds may be contacted with cells expressing the receptor polypeptide of the present invention. A second messenger response, e.g., signal transduction, pH changes, or changes in calcium level, is then measured to determine whether the potential compound activates or inhibits the receptor.

Another method involves screening for receptor inhibitors by determining inhibition or



stimulation of receptor-mediated cAMP and/or adenylate cyclase accumulation. Such a method involves transfecting a eukaryotic cell with the receptor of this invention to express the receptor on the cell surface. The cell is then exposed to potential antagonists in the presence of the receptor of this invention. The amount of cAMP accumulation is then measured. If the potential  
5 antagonist binds the receptor, and thus inhibits receptor binding, the levels of receptor-mediated cAMP, or adenylate cyclase, activity will be reduced or increased. Another method for detecting agonists or antagonists for the receptor of the present invention is the yeast based technology as described in U.S. Patent No. 5,482,835.

## 10 **Prophylactic and Therapeutic Methods**

This invention provides methods of treating abnormal conditions such as: infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; diabetes, obesity; anorexia; bulimia; Parkinson's disease; acute heart failure; hypotension; hypertension; urinary  
15 retention; osteoporosis; angina pectoris; myocardial infarction; ulcers; benign prostatic hypertrophy; leukopenia; malignant lymphomas; Hodgkin's disease; leukemias; leukocytosis; lymphadenitis; myeloproliferative disorders; myelodysplastic syndromes; myelomas; macroglobulinemia; heavy-chain disease; Langerhan's cell histiocytosis; splenomegaly; anemias; bleeding disorders; chronic obstructive pulmonary disease  
20 (COPD)-emphysema; allergies; bronchitis; asthma; bronchiectasis; adult respiratory distress syndrome; pleural effusions; pneumoconioses; sarcoidosis; systemic lupus erythematosus; Sjögren's syndrome; scleroderma; inflammatory myopathies; mixed connective tissue disease; amyloidosis; and psychotic and neurological disorders, including anxiety, schizophrenia, manic depression, delirium, dementia, severe mental  
25 retardation and dyskinesias, such as Huntington's disease or Gilles de la Tourette's syndrome, related to both an excess of and insufficient amounts of HDPXU17 activity.

If the activity of HDPXU17 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit  
30 activation by blocking binding of ligands to the HDPXU17, or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of HDPXU17 polypeptides still capable of binding the ligand in competition with endogenous HDPXU17

may be administered. Typical embodiments of such competitors comprise fragments of the HDPXU17 polypeptide.

In still another approach, expression of the gene encoding endogenous HDPXU17 can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of HDPXU17 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates HDPXU17, *i.e.*, an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of HDPXU17 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd. (1996). Another approach is to administer a therapeutic amount of HDPXU17 polypeptides in combination with a suitable pharmaceutical carrier.

30

#### Formulation and Administration

Peptides, such as the soluble form of HDPXU17 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable

pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100  $\mu\text{g/kg}$  of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Example 1: Mammalian Cell Expression:

The receptors of the present invention are expressed in either human embryonic kidney 293 (HEK293) cells or adherent dhfr CHO cells. To maximize receptor expression, typically all 5' and 3' untranslated regions (UTRs) are removed from the receptor cDNA prior to insertion into a pCDN or pCDNA3 vector. The cells are transfected with individual receptor cDNAs by lipofectin and selected in the presence of 400 mg/ml G418. After 3 weeks of selection, individual clones are picked and expanded for further analysis. HEK293 or CHO cells transfected with the vector alone serve as negative controls. To isolate cell lines stably expressing the individual receptors, about 24 clones are typically selected and analyzed by Northern blot analysis.

10

Example 2 Ligand bank for binding and functional assays:

A bank of over 200 putative receptor ligands has been assembled for screening. The bank comprises: transmitters, hormones and chemokines known to act via a human seven transmembrane (7TM) receptor; naturally occurring compounds which may be putative agonists for a human 7TM receptor, non-mammalian, biologically active peptides for which a mammalian counterpart has not yet been identified; and compounds not found in nature, but which activate 7TM receptors with unknown natural ligands. This bank is used to initially screen the receptor for known ligands, using both functional (*i.e.*, calcium, cAMP, microphysiometer, oocyte electrophysiology, etc, see below) as well as binding assays.

20

Example 3: Ligand Binding Assays:

Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for a receptor is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards its receptor. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell receptor sources. For these assays, specific receptor binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

30

Example 4: Functional Assay in *Xenopus* Oocytes:

Capped RNA transcripts from linearized plasmid templates encoding the receptor cDNAs of the invention are synthesized *in vitro* with RNA polymerases in accordance with standard  
5 procedures. In vitro transcripts are suspended in water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus* oocytes in response to agonist exposure. Recordings are made in  $\text{Ca}^{2+}$  free Barth's medium at room  
10 temperature. The *Xenopus* system can be used to screen known ligands and tissue/cell extracts for activating ligands.

Example 5: Microphysiometric Assays:

15 Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, CA). The CYTOSENSOR is thus capable of detecting the  
20 activation of a receptor which is coupled to an energy utilizing intracellular signaling pathway such as the G-protein coupled receptor of the present invention.

Example 6: Extract/Cell Supernatant Screening:

25 A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the 7TM receptor of the invention is also functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, *etc.*, functional screens) against tissue extracts to identify natural ligands. Extracts that produce  
30 positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

Example 7: Calcium and cAMP Functional Assays:

7TM receptors which are expressed in HEK 293 cells have been shown to be coupled functionally to activation of PLC and calcium mobilization and/or cAMP stimulation or inhibition. Basal calcium levels in the HEK 293 cells in receptor-  
5 transfected or vector control cells were observed to be in the normal, 100 nM to 200 nM, range. HEK 293 cells expressing recombinant receptors are loaded with fura 2 and in a single day > 150 selected ligands or tissue/cell extracts are evaluated for agonist induced calcium mobilization. Similarly, HEK 293 cells expressing recombinant  
10 receptors are evaluated for the stimulation or inhibition of cAMP production using standard cAMP quantitation assays. Agonists presenting a calcium transient or cAMP fluctuation are tested in vector control cells to determine if the response is unique to the transfected cells expressing receptor.

Example 8: Gs reporter gene luciferase assay for ligand discovery

15 The Gs reporter gene luciferase assay for orphan G-protein coupled receptors has been developed using a recombinant construct MREIIVIPII plasmid, which has been disclosed European Patent Application No. 0 863 214, published on September 9, 1998. This assay utilizes a luciferase gene fused to three copies of multiple response elements (MREs), one copy  
20 of cAMP response element (CRE) and the basal promoter of the vasointestinal peptide (VIP) gene to specifically monitor protein kinase A activation that results from an increase in intracellular cAMP. The system is not sensitive to activation of protein kinase C pathways. Rather, it responds to increases in cAMP mediated by endogenous and recombinant Gs-  
coupled GPCR activation with up to 120-fold increases in luciferase activity.

The assay begins when HEK 293 cells stably expressing the MREIIVIPII luciferase  
25 reporter gene were seeded at 4,500,000 per flask and incubated overnight at 37°C. The next day, solutions A (4 ug of pCDN-HDPXU17 DNA was added to 0.8 ml EMEM without serum) and B (23.1 ul Lipofectamine was mixed in 0.8 ml EMEM without serum ) were prepared, mixed together and incubated 30 min at room temperature. During the incubation, the culture  
medium was aspirated from cells and replaced with 12.5 ml serum-free EMEM. Next, the  
30 DNA and Lipofectamine mixture was added to the cells and incubated at 37°C for 6 hours. After 6 hours, 13 ml of EMEM containing 10% FBS was added to the cells and incubated overnight at 37°C. The next day, the 96-well plates were pretreated with 1x Matrixgel solution

at 50 ul/well for one hour. Then, the matrixgel solution and plate cells were aspirated at 2000-4000 cells/well. After overnight incubation at 37°C, 5-20 ul /well of the test compounds were added to the 96-well plates and incubated at 37°C. After 6 hours, the medium of each well was aspirated, 25 ul of 1xPBS without calcium and magnesium was added, the plates were frozen at  
5 -70°C. Then, the plates were allowed to thaw. Luc-lite solution (25 ul/well) was added and luciferase activity was measured by TopCount HTS.

In concentration ranges from 3-300,000nM, deoxyATP, deoxyAMP and UTP increase luciferase activity only in cells transfected with the HDPXU17 receptor. EC50 values for dATP and dAMP are 17.33uM and 105uM respectively. UTP is specific, but weak (EC50 not  
10 determined) compared with dATP and dAMP. Other deoxynucleotides such as dTTP, dGTP and dCTP have no effect on luciferase activity. In stable HDPXU17-expressing 293 cells(clone 20#) cells transiently transfected with MREIIVIP11 plasmid, similar results are obtained. EC50 values for dATP and dAMP are 4.05uM and 200uM respectively. UTP is still weak, but specific.

15 Based on these results, dATP appears to be the best ligand for HDPXU17 receptor (P2Y-like receptor).

Example 9: TaqMan data for HDPXU17 receptor:

TaqMan data was generated for the HDPXU17 receptor per the methods disclosed in  
20 Lie, *et al.*, *Current Opinion in Biotechnology* 9:43-48 (1998) and Gibson, *et al.*, *Genome Methods* 6:995-1001 (1996). The HDPXU17 receptor mRNA is detected by TaqMan in the following human tissues: lymphocytes, macrophages, brain, lung, liver, kidney, small intestine, colon, adipose, pancreas, prostate, placenta, cartilage, bone marrow. The most abundant expression is in lymphocytes and macrophages. This TaqMan data suggests that the  
25 HDPXU17 receptor, or agonists or antagonists thereof, may play a role in diseases including, but not limited to: diseases of white blood cells, lymph nodes, and spleen; red blood cell diseases; lung diseases; and auto-immune diseases.

30 All publications including, but not limited to, patents and patent applications, cited in this specification, are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The above description fully discloses the invention, including preferred embodiments thereof. Modifications and improvements of the embodiments specifically disclosed herein are within the scope of the following claims. Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. Therefore, the examples provided herein are to be construed as merely illustrative and are not a limitation of the scope of the present invention in any way. The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows.



**What is claimed is:**

1. A method for identifying agonist or antagonist of a polypeptide having at least a 95% identity to the amino acid sequence set forth in SEQ ID NO:2, said method comprising the steps of:
  - 5 (a) contacting a cell expressing on the surface thereof the polypeptide, said polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of a compound to said polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and
  - 10 (b) determining whether the compound binds to and activates or inhibits the polypeptide by measuring the level of a signal generated from the interaction of the compound with the polypeptide.
2. The method as claimed in Claim 1, wherein said method further comprises
  - 15 conducting the identification of agonist or antagonist in the presence of labeled or unlabeled as dATP, dAMP, or UTP.
3. The method as claimed in Claim 2, wherein the polypeptide is the amino acid sequence set forth in SEQ ID NO:2.
  - 20
4. A method for identifying agonist or antagonist of a polypeptide having at least a 95% identity to the amino acid sequencing set forth in SEQ ID NO:2, said method comprising the steps of:
  - 25 (a) determining the inhibition of binding of a ligand to cells that express the polypeptide on the surface thereof, or to cell membranes containing the polypeptide, in the presence of a candidate compound under conditions to permit binding to the polypeptide; and
  - (b) determining the amount of ligand bound to the polypeptide, such that a compound capable of causing reduction of binding of a ligand is an agonist or antagonist.

5. The method of Claim 4, wherein the ligand is labeled or unlabeled dATP, dAMP, or UTP.

6. The method as claimed in Claim 5, wherein the polypeptide is the amino acid sequence set forth in SEQ ID NO:2

5

## SEQUENCE LISTING

<110> Derk J Bergsma  
Jeffrey S Culp  
Wendy S Halsey  
Da-Yuan Wang  
Ganesh M Sathe

<120> Methods of Screening for Agonists and  
Antagonists of the HDPXU17 Receptor

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&lt;211&gt; 374

&lt;212&gt; PRT

&lt;213&gt; Human

&lt;400&gt; 2

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20           25           30
Pro Ile Leu Val Val Glu Phe Leu Val Ala Val Ala Ser Asn Gly Leu
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Ala Leu Tyr Arg Phe Ser Ile Arg Lys Gln Arg Pro Trp His Pro Ala
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 85 90 95  
 Tyr Gly Glu Ala Ala Cys Arg Leu Glu Arg Phe Leu Phe Thr Cys Asn  
 100 105 110  
 Leu Leu Gly Ser Val Ile Phe Ile Thr Cys Ile Ser Leu Asn Arg Tyr  
 115 120 125  
 Leu Gly Ile Val His Pro Phe Phe Ala Arg Ser His Leu Arg Pro Lys  
 130 135 140  
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 Ala Met Pro Thr Leu Ser Phe Ser His Leu Lys Arg Pro Gln Gln Gly  
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 Ala Gly Asn Cys Ser Val Ala Arg Pro Glu Ala Cys Ile Lys Cys Leu  
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 355 360 365  
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 370

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US99/28941

<b>A. CLASSIFICATION OF SUBJECT MATTER</b>														
IPC(7) : G01N 33/53, 33/566 US CL : 435/7.1, 7.2; 436/501 According to International Patent Classification (IPC) or to both national classification and IPC														
<b>B. FIELDS SEARCHED</b>														
Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/7.1, 7.2, 69.1, 252.3, 320.1; 436/501; 530/350; 536/23.5														
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched NONE														
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN/MEDLINE, WEST search terms: PY?, receptor#, human#.														
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>														
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.												
X,P	US 5,981,223 A (SATHE et al.) 09 November 1999, see entire document.	1-6												
A	COMMUNI et al. Cloning of a Human Purinergic P2Y Receptor Coupled to Phospholipase C and Adenylyl Cyclase. The Journal of Biological Chemistry. 19 December 1997, Vol. 272, No. 19, pages 31969-31973, see entire document.	1-6												
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.														
<table border="0"> <tr> <td>* Special categories of cited documents:</td> <td>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</td> </tr> <tr> <td>"A" document defining the general state of the art which is not considered to be of particular relevance</td> <td>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</td> </tr> <tr> <td>"E" earlier document published on or after the international filing date</td> <td>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</td> </tr> <tr> <td>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</td> <td>"A" document member of the same patent family</td> </tr> <tr> <td>"O" document referring to an oral disclosure, use, exhibition or other means</td> <td></td> </tr> <tr> <td>"P" document published prior to the international filing date but later than the priority date claimed</td> <td></td> </tr> </table>			* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A" document member of the same patent family	"O" document referring to an oral disclosure, use, exhibition or other means		"P" document published prior to the international filing date but later than the priority date claimed	
* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention													
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone													
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art													
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A" document member of the same patent family													
"O" document referring to an oral disclosure, use, exhibition or other means														
"P" document published prior to the international filing date but later than the priority date claimed														
Date of the actual completion of the international search 05 APRIL 2000		Date of mailing of the international search report 26 APR 2000												
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer <i>John D. ULM</i> JOHN D. ULM Telephone No. (703) 308-0196												